Rapid Isolation in Large Numbers of Intact, Viable, Individual Hair Follicles From Skin: Biochemical and Ultrastructural Characterization

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A rapid, novel method is described by which large numbers of intact, viable, individual hair follicles may be isolated from rat skin. Follicles are freed from the surrounding connective tissue by shearing, which is effected by repeated cutting with a loosely fitting pair of scissors, and collected individually under liquid using gentle aspiration. Ultrastructural analysis indicates that the follicles are sheared away from the surrounding dermis in the region of the connective tissue capsule which encircles the hair. The follicles appear viable by light and electron microscopy and, within 2 h of isolation, retain the capacity to incorporate [^{3}H]thymidine into DNA and [^{35}S]methionine into proteins as judged by autoradiography. A histologic comparison indicates that the structural integrity of follicles isolated by this new method is significantly superior to those plucked from the animal at the same time. The method affords the isolation of large numbers of hair follicles, without resort to enzyme treatments, suitable for biologic studies in the absence of other skin appendages and dermis. *J Invest Dermatol 87:768–770, 1986*

The conventional methods available for the isolation of intact hair follicles from human and animal sources are either to pluck hair from the skin using mechanical force or to microdissect follicles [1] from biopsy tissue. While these methods can provide a good source of follicular material suitable for cell culture [2], biochemical assays [3], and isolation of hair bulb components [4], they have a number of disadvantages. First, most [5], if not all, plucked follicles [6, this paper] may fracture along the hair bulb and suffer from internal cellular disruption [7] or external shear damage to the outer root sheath and connective tissue layer caused by the tearing force exerted to remove the follicle. Second, microdissection of follicles generally yields only a limited number of follicles dependent on the skill and perseverance of the operator. In order to obtain large numbers of structurally sound, viable hair follicles, including part of the outer connective tissue sheath, we have devised a novel method allowing several hundred individual follicles to be isolated with intact hair bulbs within 2 h. The method is particularly appropriate for animal tissues, as excision of skin is required.

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Abbreviations:
BM: basement membrane
C: hair cortex
DMEM: Dulbecco's modified Eagle's medium
DP: dermal papilla
HEPES: 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid
IRS: inner root sheath
OC: orthogonal collagen matrix
ORS: outer root sheath
SDS: sodium dodecyl sulfate
SEM: scanning electron microscopy
T: trichohyalin granule
TEM: transmission electron microscopy

MATERIALS AND METHODS

Isolation of Rat Hair Follicles Follicles were isolated from 5- to 13-day old rat skin by a modification of the procedures described by Lee et al [8] used in the isolation of eccrine sweat glands from human skin. A strip of skin was excised [~2–5 cm × 0.5 cm] and submerged in approximately 2 ml of isolation medium [Dulbecco's modified Eagles medium (DMEM; Gibco Ltd.) supplemented with 2 mM glutamine and 20 mM 4-(2-hydroxymethyl)-1-piperazine-ethane sulfonic acid (HEPES) pH 7.4] contained in a plastic Petri dish. The strip of skin was repeatedly cut with a loose-fitting pair of scissors for 1–3 m and the supernatant liquid removed and examined under a Wild M8 binocular dissecting microscope. Individual hair follicles were picked out using gentle suction from a peristaltic pump and transferred into fresh medium. The cutting procedure could be repeated further on the same strip of skin, allowing isolation of further follicles. Hairs that had a visibly damaged appearance were not selected for further study. Plucked follicles were isolated in clumps from the same animals using "strip wax."

Histology Plucked and "shear" isolated follicles were fixed immediately in 3% glutaraldehyde/0.1 M sodium cacodylate buffer, pH 7.0, for 1 h and washed in 0.1 M sodium cacodylate. For transmission electron microscopy (TEM), the follicles were post-fixed in 1% osmium tetroxide/0.1 M sodium cacodylate, followed by staining for 1 h with 2% aqueous uranyl acetate. The tissues were dehydrated through an alcohol series and embedded in "medium" epoxy resin (Taab). Thin sections (90 nm) were poststained with lead citrate and examined under the electron microscope. Samples were processed for scanning electron microscopy (SEM) as described [9]; follicles were dehydrated, embedded in wax, and sectioned (5 μm) for light microscopy.

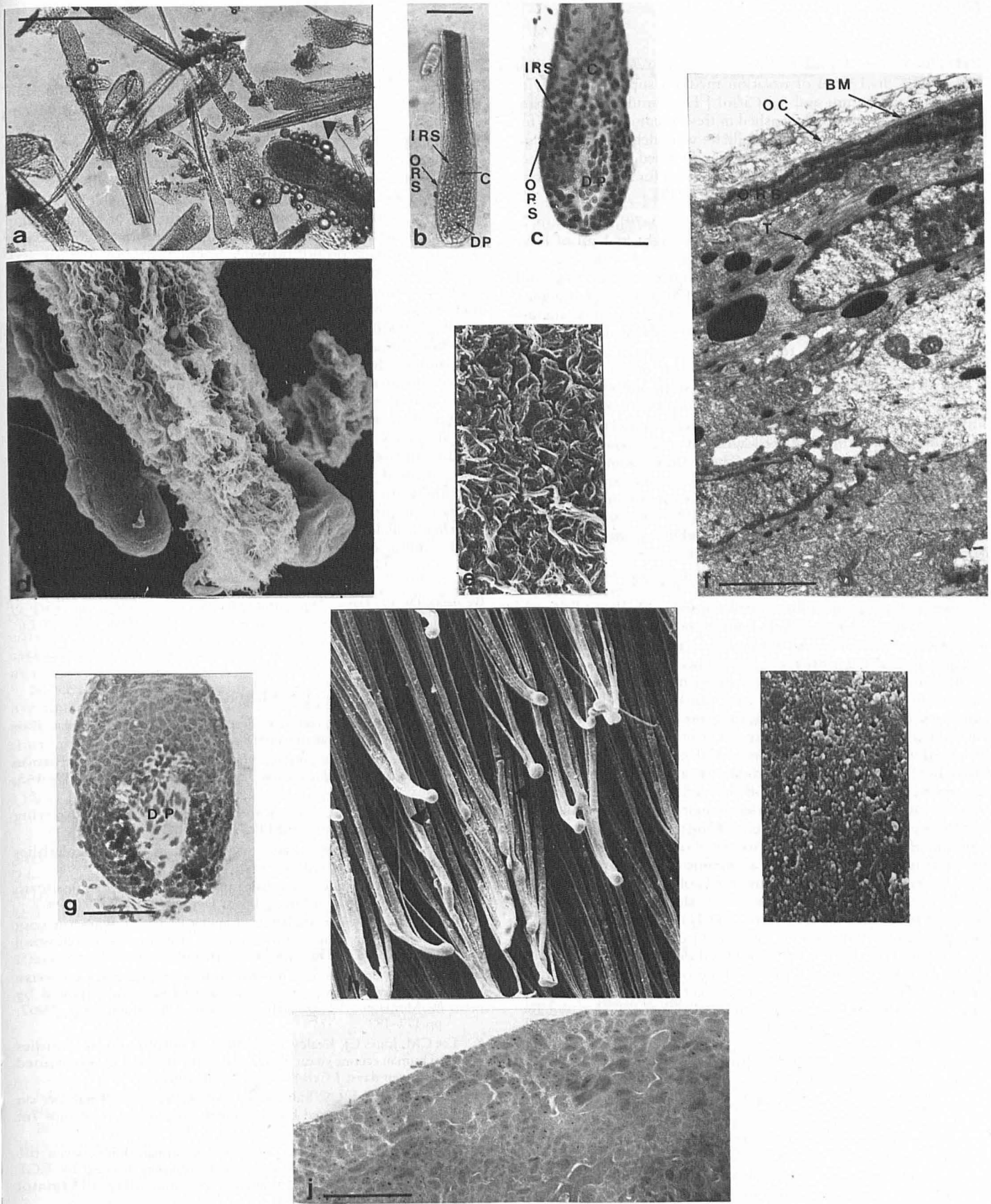

Figure 1. *a*, Phase contrast micrograph of individual follicles isolated from 13-day-old rat skin using shearing method. *Scale bar* = 200 μm. *b*, Individual follicle isolated by shearing. The major structural features of the follicle are labeled. *Scale bar* = 50 μm. *c*, Cross-section of follicle isolated by shearing, embedded in wax, and stained with H&E. *Scale bar* = 50 μm. The major structural features of the follicle are indicated. *d*, Scanning electron micrograph of follicles isolated using shearing. ×480. Some clumping has occurred during tissue processing. Follicles possess a variable amount of connective tissue which may peel away revealing an organized "smooth" fibrillar matrix. *e*, "Smooth," probably OC matrix, from a "shear" isolated follicle. ×6000. *f*, Transmission electron micrograph of "shear" isolated follicle in bulb region showing the *BM, OC, ORS,* and *T*. *Scale bar* = 0.5 μm. *g*, Autoradiograph of "shear" isolated follicle, stained with H&E, that had previously been incubated with [^{3}H]thymidine for 1 h at 37°C. Cells around the base of the DP successfully incorporate thymidine into DNA. *Scale bar* = 50 μm. *h*, Scanning electron micrograph of plucked hair follicles. ×20. The hair bulbs have an unusual rounded appearance (*arrowheads*), frequently with 2 or more structures emerging from one bulb. *i*, Scanning electron micrograph of disorganized surface matrix found on the surface of plucked hair bulbs. ×6000. *j*, Transmission electron micrograph of plucked follicle bulb, showing an absence of cellular continuity and structure. *Scale bar* = 0.5 μm. Original magnifications are given here.

[^{3}H]Thymidine Uptake Isolated follicles were incubated for 1 h at 37°C in 1.0 ml of isolation medium supplemented with 10% fetal calf serum and 5 μCi/ml [^{3}H]thymidine (Amersham plc; TRA 120), repeatedly washed in fresh medium, and fixed for 1 h in 3% glutaraldehyde. The follicles were dehydrated through an alcohol series, cleared in xylene, and embedded in wax. Sections (5 μm) were cut, processed, and stained for light microscope autoradiography as previously described [10].

[^{35}S]Methionine Uptake Hair follicles [50–70] from 5- to 7-day-old skin were incubated at 37°C overnight in 1 ml of low-methionine DMEM (Gibco Ltd.) supplemented with 1 mM sodium pyruvate, 2 mM glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 20 mM HEPES (pH 7.4), and 200 μCi [^{35}S]methionine (Amersham, SJ 123). Follicles were washed twice in low-methionine medium supplemented with 2 mM unlabeled methionine and lysed at 0°C with a total of 10 strokes in a ground glass homogenizer over 30 min in 150 μl of lysis buffer [1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM sodium chloride, 5 mM ethylenediamine tetraacetic acid, 1 mM freshly added phenylmethylsulfonyl fluoride, 150 Kallikrien units of aprotinin/ml, buffered with 50 mM Tris-HCl/30 mM sodium pyrophosphate (pH 7.4)]. The resulting homogenate was centrifuged at 10,000 *g* for 2 min in an Eppendorf microtube and the supernatant analyzed by sodium dodecyl sulfate (SDS) gradient gel electrophoresis as described [10]. The extraction procedure was designed to solubilize most cellular proteins from the hair bulb but not keratins or mature hair.

RESULTS AND DISCUSSION

A general view of hair follicles, isolated from 13-day-old skin, is shown in Fig 1*a*. Most follicle bulbs appear to be intact and to have undergone little or no mechanical damage, although some follicles are isolated with associated adipocytes (arrows, Fig 1*a*). With experience, greater than 150 intact follicles may be picked out within 2 h. However, this yield can be markedly increased (with risk of hair follicle damage) if aspiration is used to collect the isolated hairs. At higher magnification (Fig 1*b*) and in cross section (Fig 1*c*), the dermal papilla (DP), hair cortex (C), and inner (IRS) and outer (ORS) root sheaths of the hair follicles can be seen easily. By SEM, the follicles are isolated with variable amounts of residual connective tissue matrix (Fig 1*d*) which appears to peel away from the follicle, yielding a "rough" or "smooth" appearance. A higher magnification view of the smooth follicular surface (Fig 1*e*) shows an organized, fine fibrillar matrix, most probably being the layers of orthogonal collagen (OC) fibers [11] seen in cross section by TEM, covering the bulb external to the basement membrane (BM) (arrow, Fig 1*f*). Figure 1*f* also shows the ORS and trichohyalin (T) containing cells in a good state of preservation. The success of the method described here is attributed to shearing of the hair follicle along or through the external connective tissue sheath by the cutting action of the scissors.

In order to judge the viability of the isolated follicles, we assessed the uptake of [^{3}H]thymidine into DNA and the incorporation of [^{35}S]methionine into proteins using autoradiography. The isolated bulbs retained the ability to incorporate [^{3}H]thymidine into DNA, particularly by the epithelial cells lying around the base of the dermal papilla (Fig 1*g*). Hair follicles were also successfully labeled with [^{35}S]methionine with detection by SDS gradient gel electrophoresis of over 30 proteins. Major protein bands were observed at 90, 84, 68, 58, 55, 48, 44, and 30 kD (not shown). We conclude from the above biochemical and histologic data that the follicles isolated by the shearing method are complete, intact, and biochemically viable.

The ultrastructure of plucked rat follicles was also examined by SEM (Fig 1*h*) and TEM (Fig 1*j*). The majority of hair bulbs (arrowheads, Fig 1*h*; 11-day-old skin) had an abnormal, rounded appearance, suggesting damage to the hair bulb region. In addition, two or more structures were frequently seen emerging from one hair bulb, indicating substantial tissue disruption. This may represent peeling and separation of the ORS from the hair shaft and IRS components as the follicles are plucked from the skin. At higher magnification (Fig 1*i*), the surface of the plucked hair bulb had an amorphous appearance and showed no organized network of fibrils as observed with the "shear" isolated follicles. The presence of cellular damage to plucked hair bulbs was also supported by TEM (Fig 1*j*), where we were unable to detect organized tissue structure, basement membrane, or extracellular connective matrix. These data are compatible with the conclusion that plucked hairs do not provide a useful starting tissue for experiments examining the hair bulb region.

Our preliminary unpublished data also indicate that the "shear" method may be used to isolate hair follicles, as well as eccrine sweat glands [8], sebaceous glands, and apocrine sweat glands [12] from human skin. However, the method suffers from the disadvantage here that excisive skin samples are required and, indeed, on small human skin biopsies containing only a few follicles, may not be superior to microdissection methods (A. G. Messenger, personal communication).

In summary, we have described a simple, rapid method for the isolation of large numbers of intact, viable hair follicles from skin. The structural integrity of the hair bulb region is far superior to that found for plucked hair follicles and the method is considerably more productive than microdissection techniques. The isolated hair follicles represent a suitable starting material for biologic studies and in vitro hair follicle experiments.

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